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KARYOTYPE COMPOSITION IN THREE CALIFORNIA ABALONES AND THEIR RELATIONSHIP WITH GENOME SIZE

CRISTIAN GALLARDO-ESCÁRATE[1]* AND MIGUEL ÁNGEL DEL RÍO-PORTILLA[2]

[1]*Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Centro de Biotecnología, Universidad de Concepción 160-C, Concepción, Chile;* [2]*Departamento de Acuicultura, Centro de Investigación Científica y de Educación Superior de Ensenada. CICESE. Km. 107 Carretera Tijuana—Ensenada, Código Postal 22860. Ensenada, B.C. México*

ABSTRACT The fundamental goal in cytogenetics is to analyze how the structure and behavior of chromosomes guarantee the conservation of the genetic information throughout the process of inheritance, and how the chromosomal variations could affect the evolutionary process. The aim of this study is to ascertain the cytogenetical relationships of three abalone species from California. Larval cells were obtained from *Haliotis corrugata, H. fulgens*, and *H. rufescens* to obtain metaphase chromosomes. Karyotype analysis showed that all studied species have a diploid number of 2 n = 36 chromosomes. However, the relationship of chromosomal arms lengths showed that *H. rufescens* has 8M + 9SM + 1ST (metacentric + submetacentric + subtelocentric) chromosome pairs, *H. fulgens* has 8M + 8SM + 2ST, and *H. corrugata* has 10M + 7SM + 1ST. Statistical analyses carried out on abalone chromosomal morphology showed that from the 18 chromosome pairs of each species, 8 pairs were similar in all three species; 3 pairs were specific to *H. rufescens*, 7 pairs to *H. fulgens*, and 2 pairs to *H. corrugata*. Chromosome relationships showed that *H. rufescens* and *H. corrugata* are cytogenetically more similar to each other than either is with respect to *H. fulgens*. We suggest that significant chromosomal rearrangements occurred during the evolution of Haliotidae on the California coast. The implications of the karyological composition of California abalone and their genome sizes are discussed.

KEY WORDS: karyotypes, chromosome homeology, California abalone, *Haliotis corrugata*, *Haliotis fulgens*, *Haliotis rufescens*, genome size

INTRODUCTION

California abalone are marine gastropods of the genus *Haliotis* that have occupied the rocky intertidal and subtidal areas of the Pacific coast from Alaska to Baja California for at least 65 million years (Geiger & Groves 1999). Since human occupation of North America, these molluscs have been important to the lifestyle and economy of all Pacific coast populations. Native Americans valued the abalone, using the meat as a source of food and the shell for implements, trade material and decoration (Cox 1962). However, in the mid-twentieth century, California abalone populations had progressively declined because of fishing pressure and disease (Tegner et al. 1989, Celis-Ceseña 1996, McBride 1998, Burton & Tegner 2000, Hamm & Burton 2000). Nowadays, six abalone species inhabit the northeastern Pacific coast; (*H. corrugata, H. cracherodii, H. fulgens, H. rufescens, H. sorenseni*, and *H. walallensis*); besides, two abalone subspecies have been reported as *H. kamtschatkana kamtschatkana* and *H. kamtschatkana assimilis* (Leighton & Lewis 1982, Geiger & Poppe 2000, Leighton 2000).

Phylogenetic relationships among California species were initially analyzed by hemocyanin immunoassay where the degree of interaction between antigens and antibodies was measured (Meyer 1967). At present, the main approach to inferring the phylogenetic relationships has been based on DNA sequences of sperm lysin protein (Lee & Vacquier 1992, Lee & Vacquier 1995) and the vitelline egg receptor for lysin (VERL) (Swanson & Vacquier 1998, Swanson et al. 2001). Most recently, DNA-based phylogenetic studies were performed using sequencing the nuclear rDNA internal transcribed spacer (ITS) from 19 species of haliotids around the world. The ITS analysis showed that three subclades of *Haliotis* species appear consistently, each encompassing little variation. These subclades comprise the North Pacific, European, and Australian species. Within the northeastern Pacific species, the ITS analysis was congruent with the based on the sperm lysin and VERL DNA sequencing in the California abalone (Coleman & Vacquier 2002).

In reference to cytogenetic studies, several authors have proposed that speciation frequently occurs when a population becomes fixed for one or more chromosomal rearrangements, which could reduce fitness when they are heterozygous. Genetic theories, on the other hand, stress the importance of accumulation of gene mutations in reproductive isolation. Recent findings on the effects of chromosomal rearrangements on recombination have bridged the gap between the chromosomal and genetic theories of reproductive isolation, arguing for a major role for chromosomal changes in speciation (Rieseberg 2001, Navarro & Barton 2003). However, the extent of chromosomal change and subsequent speciation in marine gastropods have only been studied in a few cases (Thiriot-Quiévreux 1990, Pascoe & Dixon 1994, Thiriot-Quiévreux 1994, Pascoe et al. 1996, Amar 2003, Pascoe et al. 2004). In California abalone, because they are sympatric broadcast spawners the mechanisms of reproductive isolation are particularly interesting; including differences of bathymetrical distribution among the abalone species (Leighton 2000), and recognition species-specific of fertilization proteins (Metz et al. 1998). Chromosomal studies may provide a unique perspective on the evolution of these marine gastropods. To date, no chromosomal relationships among California abalone have been proposed yet. In fact, chromosomal data have only been reported for the black abalone *H. cracherodii* (Minkler 1977), red abalone

*Corresponding author. E-mail: cristian.gallardo@udec.cl

H. rufescens (Gallardo-Escárate et al. 2004), yellow abalone *H. corrugata*, and blue abalone *H. fulgens* (Gallardo-Escárate et al. 2005b). Available chromosome number and karyotype data indicate that California abalone has a diploid number of 36 chromosomes. However, the karyotype composition shows morphological variations for each abalone species; black abalone (8M + 8SM + 2T), red abalone (8M + 9SM + 1ST), yellow abalone (10M + 7SM + 1ST), and blue abalone (8M + 8SM + 2ST). Furthermore, the homology between chromosomes has been established from NOR-bearing data available by FISH for these species except for black abalone (Gallardo-Escárate et al. 2005a, Gallardo-Escárate et al. 2005b). All three species share two NORs, one of them in the chromosome 4, and the second one vary for each species.

In addition to the phylogenetics relationships among California abalones, mainly focused in genetic markers, the comparative study of possible chromosomal rearrangements allows to observe if the changes at molecular level are evidenced on the karyotype composition. The aims of the present study are to compare the karyotypes of three California abalone species and to analyze whether the reduction or increase of chromosomal types (metacentric, submetacentric, etc) has been accompanied by a similar variation in genome size.

MATERIAL AND METHODS

Abalone Collection and Chromosome Preparation

Three species of *Haliotis* were included in this study: the red abalone *H. rufescens* Swainson, 1822; the yellow abalone *Haliotis corrugata* Wood 1828; and the blue abalone *Haliotis fulgens* Philippi 1845. Specimens of red abalone were obtained from the Aquaculture Department, CICESE. The yellow and blue abalone were collected from a subtidal population in Cedros Island, Baja California, Mexico (28°03′N; 115°8′W) by diving. Chromosome preparations were performed from larvae according to Gallardo-Escárate et al. (2004). Briefly, trochophore larvae at 20 h postfertilization were maintained in a 0.005% colchicine solution for 3 h. Then the larvae were rinsed in clean seawater and immersed in a hypotonic solution (seawater: distilled water, 1:1) for 45 min. Finally, the larvae were fixed in modified Carnoy solution (methanol: acetic acid, 3:1). Chromosome spreads were obtained by dissociating larva tissue in acetic acid (50%), pipetting suspension drops onto slides preheated to 45°C and air dried. The slides were then washed in three changes of Phosphate Buffer Saline (1 × PBS: 13 mM NaCl, 0.2 mM KCl, 0.8 mM Na2HPO4, 0.2 mM KH2PO4, pH 7.4) for 5 min each time, and incubated with DAPI solution in the dark for 25 min at room temperature (25°C) using a coplin jar. The DAPI solution stain was prepared with 4,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, USA) in 1 × PBS at 0.5 μg/mL.

Karyotyping by Image Analysis

Chromosome spreads were karyotyped from fluorescent digital images. The best metaphases ($n = 20$) were captured using a motorized epifluorescent microscope (Leica DMRXA2) equipped with a digital camera (Leica DC300). Identification of homologous chromosomes was carried out by a karyo-ideogram performed according to Spotorno (1985). The arm lengths (short and large) were measured with Image-Pro Plus software (Media Cybernetics). Additionally, the chromosomal measurements were used to determine both relative lengths and centromeric index according to Levan et al. (1964).

Chromosomal Relationships

Chromosome-level homeology was ascertained by comparison of relative lengths of short (%SA) and long arms (%LA) of each chromosome pair. The differences between both arms were independently tested for each chromosome pair using one-way ANOVA testing and *a posteriori* Tukey testing for multiple comparisons. Before concluding statistical analysis, the percentage data were transformed to arcsine square roots to avoid distributional restrictions (Zar 1999). Thus, one chromosome pair was considered equal between two species, if both arms sizes showed no significant difference ($P > 0.05$). The Tukey test was used to build a chromosome homeology matrix species-species, where the number of chromosomes with equal morphology was obtained. Finally, a graphical approach by UPGMA clustering was carried out from similitude matrix results according to Amar (2003).

Genome Size Estimation

Nuclear DNA content or C-value (picograms of DNA per haploid genome) was determined by fluorescence image analysis (Gallardo-Escárate et al. 2005c). Briefly, this method to estimate genome size is based on the fluorescent decay lifetime (bleaching) when the fluorochrome - DNA complex is exposed to light excitation. Thus, the area under the curve during the bleaching period represents the nuclear DNA content. For the C-value analysis, we used nuclei from spermatozoa obtained by induced spawning in adult organisms. The cell suspension was smeared and fixed on clean slides with fresh Carnoy solution at 4°C and air-dried. Slides with fixed cells were washed in three changes of 1 × PBS for 5 min each, and incubated with 0.5 μg/mL DAPI solution in 1 × PBS in the dark for 25 min at 25°C. Fluorescent images were captured using QWIN software (Imaging Systems Ltd, Cambridge, UK, 1997) using a motorized epifluorescent microscope Leica DMRXA2. The image analysis was performed by an algorithm specifically programmed in MATLAB software (MathWorks, Inc.) according to Gallardo-Escárate et al. (2005c, 2007).

To assess the intraspecific and interspecific variation in genome size, a one-way ANOVA was carried out to estimate the percentage of variation at each sampling level (individuals and species) and its contribution to the total genome size variation. The coefficient of variation was calculated by the mean square × 100 from the ANOVA table. The ANOVA assumptions of normal distributions and homogeneity of variances were tested using the Kolmogorov-Smirnov and Bartlett tests, respectively. The above statistical analyses were carried out using Statistica 6.1 software (StatSoft, Inc.).

RESULTS

Karyotype of H. corrugata

Metaphases examined in the yellow abalone showed a chromosome number of 2n = 36. The karyotype consisted of 10 metacentric pairs (1, 3, 6, 9, 10, 11, 14, 15, 17 and 18), 7 submetacentric pairs (2, 4, 5, 7, 8, 12 and 13), and 1 pair

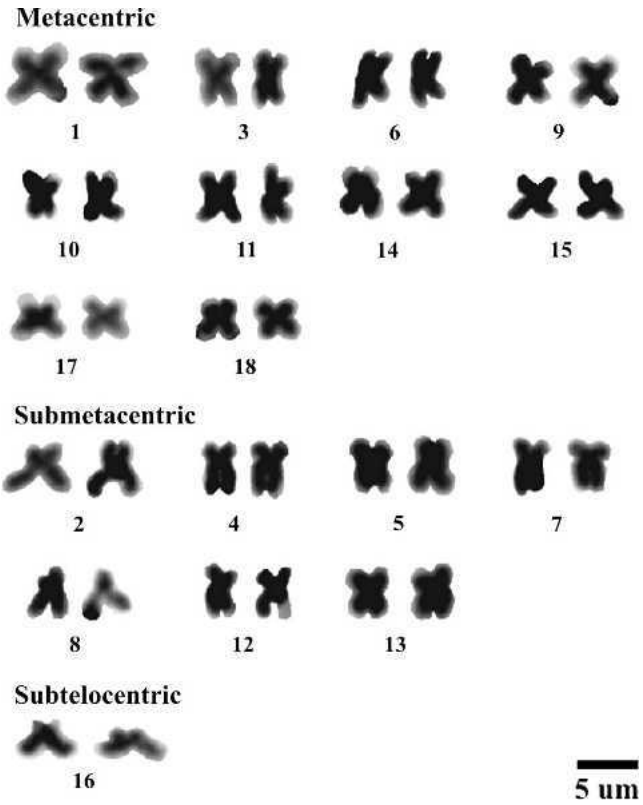

Figure 1. Karyotype of *Haliotis corrugata* (2n = 36).

of subtelocentric chromosomes (16) (Fig. 1). According to chromosome relative lengths and centromeric index from trochophore larvae, the maximum chromosome relative length was 7.28 ± 0.27 μm and the minimum was 4.32 ± 0.22 μm (Table 1). The total haploid length was 79.92 ± 0.71 μm.

***Karyotype of* H. rufescens**

All the metaphase plates analyzed showed a diploid complement of 2n = 36 chromosomes. The karyotype is composed of eight metacentric pairs (1, 3, 6, 7, 14, 15, 17, and 18), nine submetacentric pairs (2, 4, 5, 8, 9, 10, 11, 12 and 13), and one pair of subtelocentric chromosomes (16) (Fig. 2). The mean values and standard deviations of relative length and centromeric index were estimated from chromosome arm lengths. The maximum relative length of the chromosomes was 7.13 ± 0.14 μm and the minimum was 4.39 ± 0.27 μm (Table 1). The total haploid length was 77.78 ± 0.15 μm.

***Karyotype of* H. fulgens**

Metaphase chromosomes of the blue abalone showed a diploid number of 36 chromosomes. The mean and standard deviations of both relative lengths and centromeric index are shown in Table 1. The maximum relative length of the chromosomes was 8.00 ± 0.27 μm and the minimum was 4.13 ± 0.22 μm. The ratio of the chromosome arms indicated that this species has eight metacentric pairs (1, 3, 6, 9, 10, 16, 17, and 18), eight submetacentric pairs (2, 4, 5, 7, 8, 11, 12, and 13), and two pairs of subtelocentric chromosomes (14 and 15) (Fig. 3). The total haploid length was 75.89 ± 0.58 μm.

Statistical Comparisons Among Chromosomes of California Abalone Species

The 18 chromosome pairs of each abalone species were presented according to their respective morphological measurements, and classified in accordance with their centromeric position. Table 2 shows the statistical chromosome comparisons among chromosome of the three abalone species. Comparisons were performed considering all possible combinations, and one pair was considered similar between two abalone species if both chromosome arms did not show a significant

TABLE 1.

Karyotype comparison obtained among three California abalone species. RL, relative length; CI, centromeric index; M, metacentric; SM, submetacentric; and ST: subtelocentric.

Pair	RL ± std			CI ± std		
	Corrugata	*Rufescens*	*Fulgens*	*Corrugata*	*Rufescens*	*Fulgens*
1	7.28 ± 0.27	7.13 ± 0.14	8.00 ± 0.27	48.02 ± 0.06 (M)	49.50 ± 0.29 (M)	47.45 ± 0.16 (M)
2	6.26 ± 0.12	6.70 ± 0.05	6.67 ± 0.12	35.90 ± 0.11 (SM)	33.35 ± 0.11 (SM)	34.09 ± 0.15 (SM)
3	6.03 ± 0.38	6.56 ± 0.12	6.37 ± 0.38	46.70 ± 0.05 (M)	47.16 ± 0.25 (M)	48.37 ± 0.17 (M)
4	6.00 ± 0.49	6.33 ± 0.20	6.23 ± 0.49	39.95 ± 0.08 (SM)	37.46 ± 0.40 (SM)	39.81 ± 0.18 (SM)
5	5.74 ± 0.22	6.22 ± 0.11	5.48 ± 0.22	36.93 ± 0.06 (SM)	37.61 ± 0.21 (SM)	34.16 ± 0.14 (SM)
6	5.66 ± 0.12	6.15 ± 0.04	6.08 ± 0.12	43.81 ± 0.09 (M)	49.42 ± 0.07 (M)	44.69 ± 0.13 (M)
7	5.66 ± 0.07	6.01 ± 0.07	6.01 ± 0.07	34.51 ± 0.09 (SM)	49.75 ± 0.15 (M)	39.41 ± 0.10 (SM)
8	5.57 ± 0.40	5.72 ± 0.17	5.82 ± 0.40	36.91 ± 0.08 (SM)	37.96 ± 0.34 (SM)	37.27 ± 0.17 (SM)
9	5.55 ± 0.20	5.60 ± 0.17	5.59 ± 0.20	49.16 ± 0.07 (M)	38.64 ± 0.34 (SM)	42.74 ± 0.15 (M)
10	5.43 ± 0.10	5.45 ± 0.07	5.45 ± 0.10	41.09 ± 0.07 (M)	34.08 ± 0.14 (SM)	43.77 ± 0.12 (M)
11	5.40 ± 0.20	5.15 ± 0.19	5.32 ± 0.20	47.98 ± 0.16 (M)	35.41 ± 0.39 (SM)	37.98 ± 0.15 (SM)
12	5.36 ± 0.26	5.06 ± 0.12	5.24 ± 0.26	37.38 ± 0.04 (SM)	36.38 ± 0.23 (SM)	34.51 ± 0.09 (SM)
13	5.30 ± 0.31	4.87 ± 0.06	5.02 ± 0.31	34.82 ± 0.08 (SM)	34.33 ± 0.13 (SM)	40.59 ± 0.15 (SM)
14	5.28 ± 0.21	4.82 ± 0.06	5.09 ± 0.21	44.74 ± 0.10 (M)	48.66 ± 0.11 (M)	24.73 ± 0.14 (ST)
15	5.09 ± 0.25	4.75 ± 0.05	4.78 ± 0.25	47.04 ± 0.03 (M)	48.75 ± 0.10 (M)	24.82 ± 0.17 (ST)
16	5.04 ± 0.19	4.64 ± 0.02	4.61 ± 0.19	22.44 ± 0.04 (ST)	27.40 ± 0.05 (ST	44.97 ± 0.12 (M)
17	5.02 ± 0.23	4.47 ± 0.07	4.13 ± 0.23	48.59 ± 0.02 (M)	49.77 ± 0.14 (M)	48.29 ± 0.20 (M)
18	4.32 ± 0.22	4.39 ± 0.27	4.13 ± 0.22	45.50 ± 0.07 (M)	49.97 ± 0.55 (M)	45.20 ± 0.09 (M)

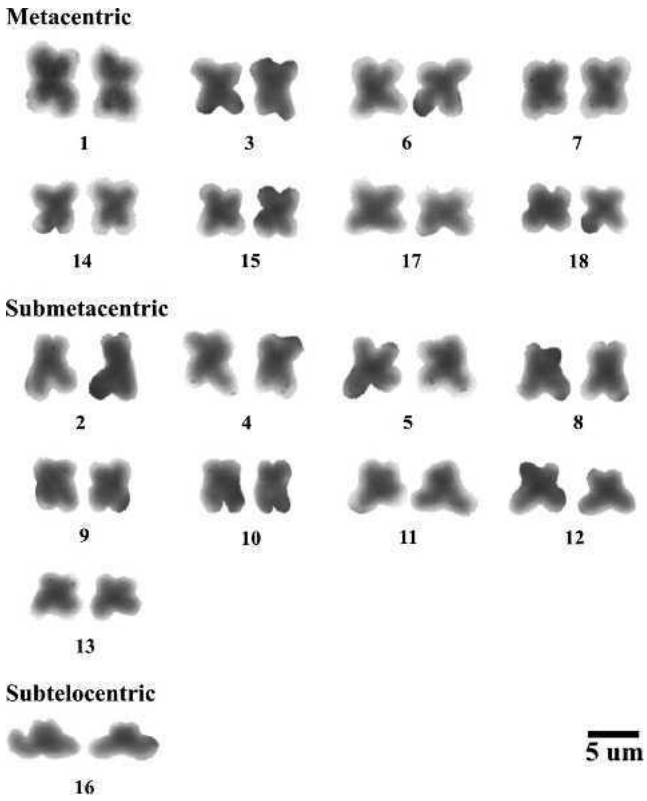

Figure 2. Karyotype of *Haliotis rufescens* (2n = 36).

difference. These analyses showed there were no statistical differences among the species for chromosome pairs 1, 2, 3, 4, and 5. Likewise, the chromosome types did not differ among the three abalones species studied (Table 1). Chromosome pair 6 was metacentric in all studied species, but a major difference in chromosomal length was observed in the red abalone for this chromosome pair. Chromosome 7 was submetacentric in both yellow and blue abalone, but metacentric in the red abalone. Chromosome 8 was classified as a submetacentric type in all three abalone species. However, the blue abalone showed significant differences ($P < 0.05$) in length in relation the other species. Chromosomes 9, 10, and 11 were found mainly as the metacentric type, with the exception of the red abalone, in which it was a submetacentric type. The statistical analyses showed that these chromosomes were the most variable in length among the studied abalone. Furthermore, the three California species had both chromosomes 12 and 13 of the submetacentric type. Chromosomes 14 and 15 were classified as metacentric in the yellow and the red abalone, whereas in blue abalone they were classified as subtelocentric. Likewise, the greatest differences were found in this species. Chromosomes pair 16 was described as subtelocentric in the red and yellow abalone, with blue abalone being the exception, classified as metacentric. Finally, chromosome 17 and 18 did not show statistical differences in length among species, and were also classified as the metacentric type. In conclusion, the statistical analyzes showed that of the 18 chromosome pairs described earlier, 8 pairs were conserved among the California abalone, 3 chromosomes were characteristic to the red abalone (*H. rufescens*), 7 chromosomes to the blue abalone (*H. fulgens*), and 2 chromosomes to the yellow abalone (*H. corrugata*) (Table 2). In addition, the chromosomal similitude matrix shows that *H. rufescens* shares 9 chromosome pairs with *H. fulgens*, and 14 chromosome pairs with *H. corrugata*. Likewise, between *H. fulgens* and *H. corrugata*, 8 chromosome pairs were shared (Table 3). Additionally, from the species by species matrix the UPGMA showed that *H. rufescens* and *H. corrugata* are more similar than either are to *H. fulgens* (Fig. 4).

Genome Size of California Abalone

The results of image analysis to determine the genome size using spermatozoa of Californian abalone, showed that the yellow abalone had 2.14 ± 0.45 pg ($n = 399$), the red abalone had 1.82 ± 0.36 pg ($n = 264$), and the blue abalone had 1.71 ± 0.33 pg ($n = 347$) of nuclear DNA. Figure 5 shows the genome size variation of the three studied abalone species. The use of image analysis allowed the analysis of a large numbers of cells, and thereby the determination of accurate values of nuclear DNA contents. The statistical analysis of abalone genome size showed that the interindividual variation was less than 15% (coefficient of variation). The analysis of variance performed to detect differences at species level, showed significant differences among the three abalones species ($P < 0.05$). The genome size of the *H. corrugata* was statistically larger compared with the other species. The smallest genome was found in the *H. fulgens*, whereas the *H. rufescens* genome was located in midway.

DISCUSSION

Our results confirmed a characteristic diploid number of 2n = 36 chromosomes in California abalone so far studied, and we can summarize the results as follows: (1) the karyotype of

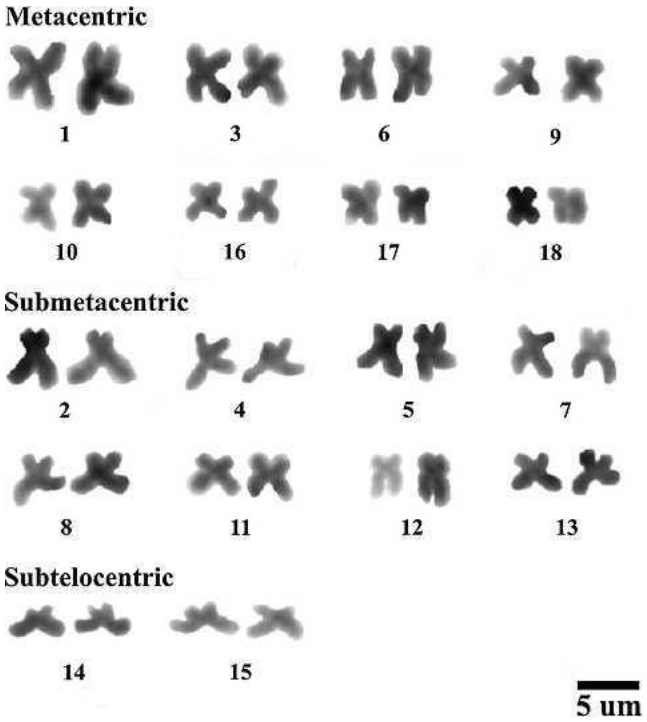

Figure 3. Karyotype of *Haliotis fulgens* (2n = 36).

TABLE 2.

Chromosomal comparison results among three California abalone species. For each pairwise comparison, the chromosome homeology was tested between short (SA) and long (LA) arms. The chromosome pair was considered as conserved (=) among the three species if arm lengths show no statistical significance. Contrarily, they were considered different (*); (R) chromosome pair exclusive to *H. rufescens*, (F) chromosome pair exclusive to *H. fulgens*, (C) chromosome pair exclusive to *H. corrugata*.

	H. rufescens x *H. corrugata*		*H. corrugata* x *H. fulgens*		*H. fulgens* x *H. rufescens*		
Pair	**SA**	**LA**	**SA**	**LA**	**SA**	**LA**	**Result**
1	–	–	–	–	–	–	=
2	–	–	–	–	–	–	=
3	–	–	–	–	–	–	=
4	–	–	–	–	–	–	=
5	–	–	–	–	–	–	=
6	*	–	–	–	*	–	R
7	*	*	*	–	–	–	R
8	–	–	*	–	*	*	F
9	*	–	*	–	*	–	R, C and F
10	–	–	*	–	*	–	F
11	*	–	*	*	–	–	C
12	–	–	*	–	*	*	F
13	–	–	*	*	–	*	F
14	–	–	*	*	*	*	F
15	–	–	*	*	*	*	F
16	–	–	*	–	*	–	F
17	–	–	–	–	–	–	=
18	–	–	–	–	–	–	=

(–) No statistical difference ($P > 0.05$).
(*) Statistical difference ($P < 0.05$).

yellow abalone *H. corrugata*, has a 10M + 7SM + 1ST (metacentric + submetacentric + subtelocentric), which has the greatest proportion of metacentric chromosomes, and the lowest number of submetacentric chromosomes. Total haploid length and genome size were found to be the greatest among all the studied species. (2) The red abalone *H. rufescens* showed a karyological conformation of 8M + 9SM + 1ST, and the total haploid length as well as genome size was located midway between yellow and blue abalone. (3) The lowest total haploid length and genome size was found in the blue abalone *H. fulgens*. This abalone species also showed an increase in the proportion of subtelocentric chromosomes (8M + 8SM + 2ST) compared with both red and yellow abalone. These results and the statistical analyses carried out on abalone chromosomal morphology, showed that from the 18 chromosome pairs of each species, 8 pairs were similar in the three studied species; 3 pairs were specific to *H. rufescens*, 7 pairs to *H. fulgens*, and 2 pairs to *H. corrugata*. UPGMA relationships based on chromosome data showed that both *H. rufescens* and *H. corrugata* are cytogenetically more similar than either are to *H. fulgens*. The comparative values from this study suggest a positive correlation between genome size and total haploid length, and in agreement with an increase in the proportion of submetacentric-subtelocentric chromosome types within of the karyotypes studied. This correlation would suggest that no changes in the degree of DNA coiling and/or folding must have occurred. To achieve this, according to Summer (1990) and Pascoe et al. (2004), the packing ratio (length of naked DNA/length of assembled DNA) could be calculated by using the mammalian packing ratio approach. The haploid genome of *Homo sapiens* amounts to about 3 pg of DNA, which is equivalent to, approximately 1-m length of DNA. The total haploid length for human chromosomes is approximately 100 μm so the packing ratio is 10,000. For the abalone studied here the packing ratio of *H. corrugata* is 9,050, and 7,799 and 7,510 for *H. rufescens* and *H. fulgens*, respectively. Furthermore, the genome sizes calculated in this study for the California abalone are congruent with the reported values by Hinegardner (1974).

Chromosomal rearrangement observed in California abalone could be supported by the physical localization of genes using fluorescence *in situ* hybridization. In this context, we recently used FISH analysis with ribosomal probes to locate rDNA 18S-5.8S-28S clusters, which form the nucleolus organizing regions (NORs) (Gallardo-Escárate et al. 2005a, Gallardo-Escárate et al. 2005b). These studies showed that in California abalone two major rDNA clusters are terminally located on the telomeric regions of the large chromosome arms. One of them was found in all the studied abalone species on chromosome 4, whereas a second NOR was observed on chromosome 2 of *H. corrugata,* chromosome 5 of *H. rufescens*,

TABLE 3.

Chromosome homeology matrix obtained among chromosomes of *H. rufescens*, *H. fulgens* and *H. corrugata*. Values are number of identical chromosomes shared.

	H. corrugata	*H. rufescens*	*H. fulgens*
H. corrugata	*	14	8
H. rufescens		*	9
H. fulgens			*

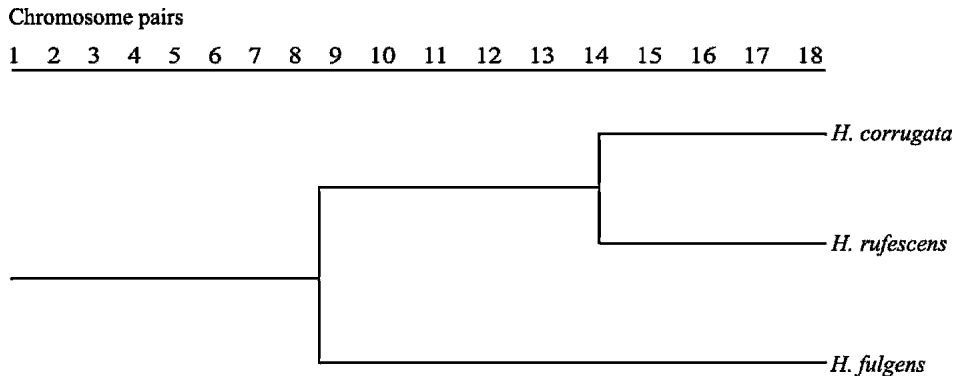

Figure 4. Cluster by UPGMA analysis from chromosome homeology matrix. The scale shows the number of chromosome pairs shared among *H. corrugata*, *H. rufescens*, and *H. fulgens*.

and chromosome 11 of *H. fulgens*. All these chromosomes are submetacentric; however, the specific localization shows that the NOR located in pair 11 of *H. fulgens* was the shortest chromosome among the studied species with rDNA clusters. To establish a hypothesis about the chromosomal evolutionary trend of California abalone, it is imperative joined our results with the available molecular data. Thus, several studies have been carried out to approach the phylogenetic characteristics of abalones throughout the world (Lee & Vacquier 1992, 1995, Swanson & Vacquier 1998, Swanson et al. 2001, Coleman & Vacquier 2002, Streit et al. 2006). Although in general it is possible to find agreement, there are somewhat different relationships with respect to California abalones. For instance, Lee & Vacquier (1995) describe one main group of closely related species, and comprising of *H. rufescens, H. sorenseni, H. walallensis* and *H. kamtschatkana*. With respect to

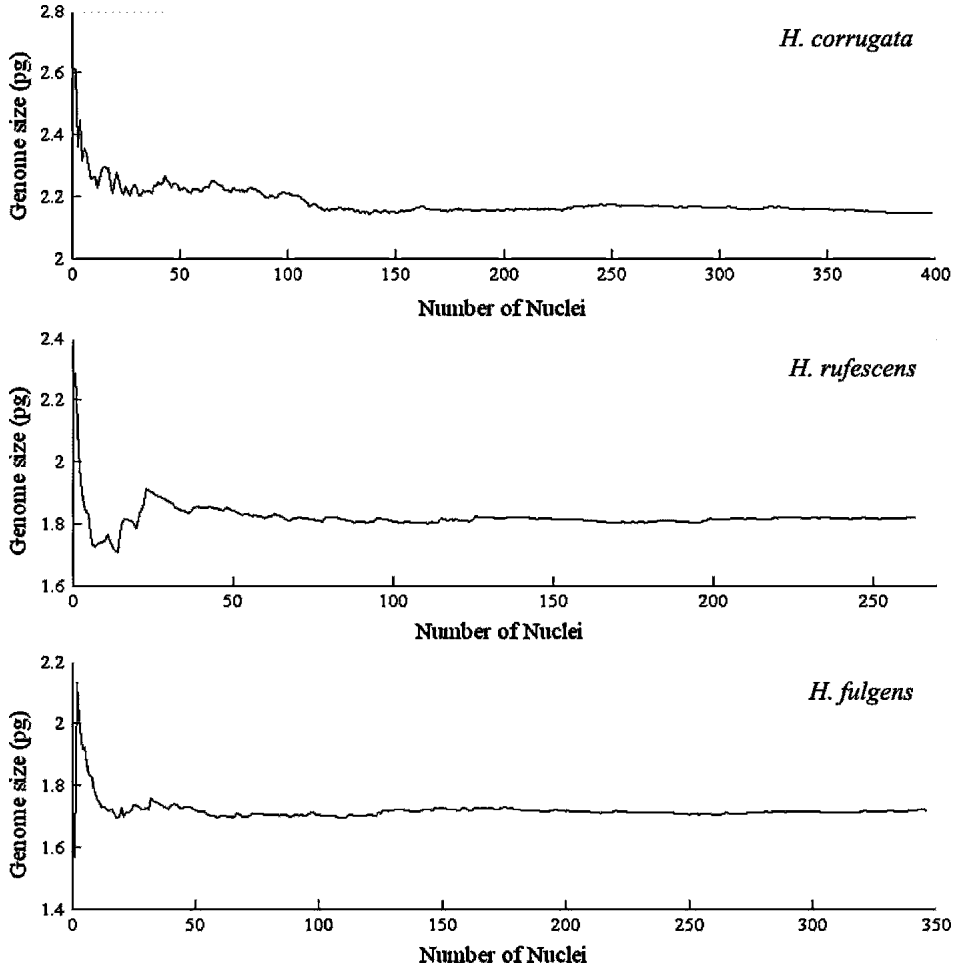

Figure 5. Genome size variations found in *H. corrugata*, *H. rufescens*, and *H. fulgens*. The genome size was calculated by fluorescence image analysis. The mean genome size was plotted by increases from 2 to *n* spermatozoa nuclei.

H. corrugata, this species appeared moderately distinct from the main group, whereas *H. fulgens* and *H. cracherodii* were decidedly different from the other California abalone species. An important fact was that *H. corrugata* appeared more related to Japanese abalone as *H. discus hannai*. On the other hand, Streit et al. (2006) report for the North Pacific clade that *H. fulgens* and *H. corrugata* are basal species with respect to *H. rufescens*, and they are closely related. Moreover, the Japanese abalone *H. discus hannai* appears more closely related to *H. rufescens* than the other two abalone species. In this scenario, ours results could only be in agreement with Lee & Vacquier (1995), but it is not possible to give support under the Streit el at. (2006) approach. In this context, the tendency obtained by UPGMA is to increase the DNA content (1.71; 1.82; 2.14 pg), and to increase the chromosome length (75,89 in the basal *H. fulgens*; 77,78 and 79,92 in *H. rufescens* and *H. corrugata* respectively).

In reference to the evolution of other groups, there appear to be no general rules with regards to genome size and chromosomal rearrangements. In various invertebrate groups studied, no general evolutionary trend in chromosome number has been evident. In gastropods caution is even advised in interpreting chromosome number as an indicator of evolutionary relationships (Thiriot-Quiévreux 1994, 2003). However, Pascoe et al. (2004) reported that members of the Muricidae in the northern hemisphere show striking variation in chromosome number. The majority of these neogastropods have chromosome numbers in the range $2n = 60–70$, whereas the Atlantic dog-whelk *Nucella lapillus*, is unique in having a Robertsonian polymorphism with 2n values in the range of 25–36 chromosomes. In this context, changes in the proportion of heterochromatin and packing ratios of the metaphase chromosomes are involved. The evolution in *N. lapillus,* the derived form within the group, appears to have involved a reduction in chromosome number and haploid chromosome length, but an increase in both genome size and packing ratio. Contrary to this, our results in abalone show that the evolutionary trend appears to be an increase in chromosome number as well as in the proportion of metacentric-submetacentric chromosome.

Abalone (family Haliotidae) are widely distributed in the world's oceans and are located along most rocky shores in tropical and temperate waters, and are mostly encountered in the shallow subtidal region between sea level and a 30 m depth. This substrate requirement could be explain the absence of haliotid representatives from northeastern America as well as in the western Indian coasts. Abalone are also not known from polar regions and western South America (Geiger & Poppe 2000). This geographical distribution plus the occurrence of discrete regions of endemism have encouraged postulating several hypothesis about the biogeographic origin of Haliotidae. According to Geiger and Groves (1999), the *Pacific Rim model*, explains that an arc spanning from Japan to northeastern Australia has been identified as a likely cradle of the family. From this nuclear distribution, abalone then dispersed in a star shaped pattern to the northwestern and then northeastern Pacific, to Australia and the Indian Ocean. The *Indo-Pacific model* is based on the highest present-day diversity of the family, which is found in the Indo-Malayan area. Although it is well appreciated that the correlation of high present day diversity with the origin of the group in question is very problematic, it provides one possible center of radiation for the family. The *Tethys model* is based on published chromosomal data. The review of karyotypes reported in abalone shows a variation of diploid numbers varying from 28–36 chromosomes (Jarayabhand et al. 1998, Gallardo-Escárate et al. 2004). According to their geographic distribution (Geiger & Poppe 2000), the abalone from the European Mediterranean region have $2n = 28$, haliotids from the Indo-Pacific region have a characteristic $2n = 32$, with the exception of *H. aquatilis* with a diploid number equal to 34 chromosomes, and the abalone found in the South Japan region show $2n = 32$. Finally, abalone from the North Pacific as *H. discus hannai* (Arai et al. 1982) and *H. cracherodi* (Minkler 1977) have a diploid number of 36 chromosomes. In this context, if the increase in diploid chromosome number is indicative of a likely evolutionary direction could suggest that abalones from the European-Mediterranean region are relict species from the ancient Tethys Sea, and those abalones were dispersed eastwards, which is in agreement with the eastward dispersal pattern in the Pacific, being the California abalone the most recent species within the Haliotidae family. However, the previously mentioned evidence is mainly founded in morphological homologies of chromosomes that could only be supported with additional cytogenetical evidence that involves molecular markers or banding techniques. Moreover, recently data shown by Streit et al. (2006) using hemocyanin sequences establish that there are two genetically different monophyletic groups in Haliotidae with high bootstrap support; the abalone from Europe, South Africa, Australia, Taiwan and Japan group together and are separated from five California and two Japanese abalone. In this scenario, the trend inferred with chromosomal data that Haliotidae shows an increase of chromosome number because of rearrangements needs to be reviewed with additional studies.

In summary, we suggest that significant chromosomal rearrangements occurred during the evolution of haliotids presents on the California coast. However, important questions remain not solved; why should selection have favored these chromosomal rearrangements? California abalone can produce hybrids (Owen et al. 1971, Leighton & Lewis 1982), therefore how could these hybrids stabilize their chromosomal composition? The variation of genome size (e.g., repetitive mobile elements and satellite DNA) could influences the chromosomal evolutionary trends in Haliotidae?

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